BIO 101® Systems

APPLICATION MANUAL

PhoenIX™ Gigaprep Kit

Vacuum-driven ion exchange purification of ≤ 10 mg plasmid DNA from 2.5 – 5.0 L of E. coli cells

Revision # 2075-400-4A01

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Catalog # 2075-400

5 preps

Storage:

Unopened box should be stored at ambient temperature (15-30ºC).

Once RNase A has been added to Cell Resuspension Buffer, store at 4ºC and use within 6 months.

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Appendix I. PhoenIX™ Gigaprep Quick Reference Protocol

1. Introduction

PhoenIX™ plasmid purification systems from Qbiogene rely on ion-exchange chromatography through a unique, patented solid-phase column. Precisely balanced pH values and salt concentrations permit optimal purification of highly clean plasmid DNA from the unique ion-exchange resin. Unlike resins found in other ion-exchange kits, PhoenIX™ columns are distinct: First, the smaller pore size of the resin particles increases the surface area available for binding. Second, the spacer molecule linking the positively charged amino group to the resin particles is longer than in other resins, allowing a better degree of separation between different biomolecules during the binding and washing process.

The PhoenIX™ Gigaprep Kit is driven by vacuum and offers lysate clearing via a filtration cartridge. The PhoenIX™ Midiprep Kit and PhoenIX™ Maxiprep Kit rely on centrifugation to clear the bacterial lysate, and on gravity to pull liquids through the column material.

Plasmid DNA purified with PhoenIX™ columns can be used immediately in a wide variety of downstream applications including automated fluorescent sequencing, enzymatic amplification, labeling, transcription, cloning and other enzymatic manipulations. Additionally, the levels of endotoxin that remain after purification are so low that successful transfection will occur with even sensitive cell lines.

2. Kit Components and User Supplied Materials

2.1 PhoenIX™ Gigaprep Kit Components

Description	Quantity
RNase A (red cap)	82.5 mg
Cell Resuspension Buffer (yellow cap label)	650 ml
Lysis Buffer (blue cap label)	650 ml
Neutralization Buffer (green cap label)	650 ml
PhoenIX™ Giga Columns	5 each
Lysate Filtration Cartridges	5 each
Equilibration Solution (gray cap label)	1025 ml
Column Wash Buffer (orange cap label)	3 x 1010 ml
Elution Buffer (pink cap label)	525 ml
User Manual	1 each
MSDS	1 each
Certificate of Analysis	1 each

2.2 User Supplied Materials

- Isopropanol
- 70% ethanol
- Centrifuge bottles (500 ml or larger) capable of withstanding 13,000 x g for pelleting 2.5 – 5.0 liters of cell culture
- Vacuum source capable of generating a negative pressure of -20 inches Hg (-600 to -800 mbar). It is essential that a vacuum control device be placed just next to the filter bottle so that the vacuum pressure can be adjusted down to -5 to -8 inches Hg during the procedure.
- 500 ml vacuum-resistant laboratory bottle with a 45 mm neck for the collection of the cleared lysate*
- Sterile spatula for stirring bacterial lysate
- 1.0 L vacuum-resistant laboratory bottle with a 45 mm neck for the collection of equilibration, binding and washing flow-through*
- 100 – 200 ml vacuum-resistant laboratory bottle with a 45 mm neck for the elution step*
- Centrifuge bottles (500 ml) capable of withstanding 13,000 x g for precipitation of plasmid DNA

**Do not use plastic or glass bottles or any other vessels that are not designed for use with a vacuum. Bottles should not be cracked or scratched in any way. Wear safety glasses when working near a bottle under vacuum.*

3. Safety Precautions

Several components contain compounds that may cause irritation or burning when in contact with human tissue or during inhalation. Wear personal protective equipment to prevent skin contact (e.g. gloves, lab coat, and eye protection) and prevent inhalation of reagent vapors and consumption of liquid during use. Consult the enclosed Material Safety Data Sheet for additional details.

Do not use plastic or glass bottles or any other vessels that are not designed for use with a vacuum. Bottles should not be cracked or scratched in any way. Wear safety glasses when working near a bottle under vacuum.

4. Things to Know before Using the PhoenIX™ Gigaprep Kit

4.1 Read Protocol Thoroughly before Use

For optimal yield and purity of plasmid DNA, it is critical to closely follow the protocol as directed. Although the process of bacterial cell growth and lysis may be able to withstand many short-cuts, each time a step is not performed optimally the risk of an unacceptable result increases.

4.2 Growth of Bacterial Cells

Inoculate 10 ml of selective medium with a single colony from a freshly streaked selective agar plate. Incubate at least 8 hours at 37°C with vigorous shaking (~300 rpm).

Dilute the starter culture 1:500 to 1:1000. (For example, add 5 ml of a high-copy plasmid starter culture to 2.5 L of fresh selective media). Grow *E. coli* cells at 37°C overnight with vigorous shaking to a density between 1 – 5 x 10^9 cells per ml of medium (1 – 1.5 OD_{600} units/ml). For high-copy plasmids (present in concentrations of ≥4 – 5 µg/ml), do not use more than 2.5 liters of bacterial culture.

4.3 Prepare Cell Resuspension Buffer by adding RNase A

To resuspend lyophilized RNase A (red cap), add 1.0 ml of Cell Resuspension Buffer (yellow cap label) and vortex. Remove the entire volume of resuspended RNase A and add it to the bottle of Cell Resuspension Buffer. Mix well and check the "Contains RNase A" box on the label. Prepared Cell Resuspension Buffer should be stored at 4°C and used within 6 months.

Qbiogene strongly recommends preparing the entire volume of Cell Resuspension Buffer at once. However, if it is certain that all of the prepared Cell Resuspension Solution will not be used within 6 months, a partial batch can be prepared instead. Resuspend the RNase A as described above and add the appropriate amount to a smaller volume of Cell Resuspension Solution. For example, instead of adding 1 ml of resuspended RNase A to 649 ml of buffer, add 0.5 ml of resuspended RNase A to 324.5 ml of buffer. The prepared Cell Resuspension Solution should be stored at 4°C as directed above. The remaining volume of resuspended RNase A solution can be stored at -20°C for up to 2 years.

4.4 Precipitate Material in Lysis Buffer

If the PhoenIX™ Gigaprep Kit was shipped or stored at a low temperature, a harmless precipitate may form in the Lysis Buffer (blue cap label). If a precipitate is seen, incubate the bottle in a 37°C water bath for several minutes and mix to bring the precipitate back into solution.

5. Detailed Protocol

Before beginning, verify that RNase A (red cap) has been added to the Cell Resuspension Buffer (yellow cap label) and that no precipitate can be seen in the Lysis Buffer (blue cap label). See Sections 4.3 and 4.4.

1. Set up a Lysate Filtration Cartridge by attaching a clean, 500 ml bottle with a 45 mm neck.

NOTE: Do not over-tighten the Lysate Filtration Cartridge onto the bottle neck or the plastic may crack.

2. Divide culture as necessary in order to pellet 2.5 – 5.0 liters of a 1 - 5 x 10^9 cells/ml bacterial culture (see section 4.2). Centrifuge at 13,000 x g for 3 minutes at room temperature.

NOTE: For high-copy plasmids (present in concentrations of ≥4 – 5 µg/ml), do not use more than 2.5 liters of bacterial culture.

3. Remove all traces of liquid medium from the bacterial cell pellet with a pipette.

NOTE: If traces of culture medium remain with the cells, the ratio of salts and pH values will not be optimal in subsequent steps.

4. Use a total volume of 125 ml of RNase A-containing Cell Resuspension Buffer (yellow cap label) to completely resuspend and pool the cell pellets by vortexing.

5. Add 125 ml of Lysis Buffer (blue cap label) and securely close the sample bottle. Mix thoroughly by inverting until the lysate appears to be homogeneous (5-6 inversions). DO NOT VORTEX.

6. Incubate 5 minutes at room temperature.

NOTE: Do not incubate for longer than 5 minutes or plasmid DNA might become irreversibly denatured.

7. Add 125 ml of Neutralization Buffer (green cap label). Securely cap the tube and mix immediately by multiple inversions until a homogeneous suspension containing no viscous matter is obtained. DO NOT VORTEX.

NOTE: If preparing several samples at once, thoroughly mix each sample immediately after the addition of the Neutralization Buffer before adding the buffer to the next sample. A white, flocculent precipitate made of proteins, cellular debris, genomic DNA and detergent will form, which is normal. All viscous matter should be converted to a thin mixture.

8. Pour the bacterial lysate from step 7 directly into the prepared Lysate Filtration Cartridge assembled in step 1.

9. Let the bacterial lysate stand at room temperature for at least 10 minutes without agitation.

NOTE: The white, flocculent precipitate material must float up to the surface of the bacterial lysate before applying a vacuum to the cartridge.

10. Attach the vacuum source to the tubing connector on the Lysate Filtration Cartridge and apply the vacuum (-20 inches Hg). Filtration should be complete after 1-2 minutes.

11. Add 50 ml of Column Wash Buffer (orange cap label) to the Lysate Filtration Cartridge and gently stir the precipitate with a sterile spatula. Connect the vacuum source again and apply vacuum until all liquid has been pulled through completely. The bottle now contains the filtered lysate containing the plasmid DNA.

NOTE: Gentle agitation of the precipitate improves the flow of liquid through the filter unit.

12. Prepare a PhoenIX™ Giga Column by attaching a clean, 1.0 L bottle with a 45 mm neck.

13. Add 200 ml Equilibration Solution (gray cap label) to the PhoenIX™ Giga Column. Attach the vacuum source and apply the vacuum (-20 inches Hg) until all liquid has drained from the resin. Discard the flow-through and re-attach the bottle to the column.

14. Carefully pour the cleared bacterial lysate from step 11 onto the equilibrated PhoenIX™ Giga Column. Apply vacuum (-20 inches Hg) until all of the lysate has passed through the resin.

NOTE: If desired, an aliquot of the flow-through can be kept for further analysis.

15. Add 275 ml of Column Wash Buffer (orange cap label) to the PhoenIX™ Giga Column. Apply vacuum (-20 inches Hg) until all of the lysate has passed through the resin.

NOTE: If desired, an aliquot of the flow-through can be kept for further analysis.

16. (Optional) Add a second volume of 275 ml of Column Wash Buffer (orange cap label) to the Phoenix™ Giga Column. Apply vacuum (-20 inches Hg) until all of the lysate has passed through the resin.

NOTE: A second wash is necessary when a culture volume > 3.0 liters is used or when preparing plasmid DNA from a bacterial host strain that produces a large quantity of RNA or carbohydrate contaminants. For most plasmid preparations, a single wash is sufficient to remove all contaminants completely. If desired, an aliquot of the flow-through can be kept for further analysis.

17. Remove the 1.0 L bottle from the PhoenIX™ Giga Column and replace it with a clean, 100 - 200 ml bottle with a 45 mm neck.

18. Add 100 ml of Elution Buffer (pink cap label) to the column.

19. Re-attach the vacuum source and apply a soft vacuum (-5 to -8 inches Hg) to the column until 20 – 40 ml eluate has passed through the resin.

20. Remove the vacuum source from the column and let stand 1 minute at room temperature without agitation.

21. Re-attach the vacuum source and apply a soft vacuum (-5 to -8 inches Hg) to the column until the remainder of the liquid has passed through the resin.

NOTE: If desired, an aliquot of the flow-through can be kept for further analysis.

22. Add 0.7 volumes of Isopropanol to the eluted plamid DNA. Mix well and centrifuge at 12,000 x g for 30 minutes at 4°C.

23. Pour out the supernatant taking care not to disturb the DNA pellet.

24. Add 20 ml of 70% ethanol and wash the pellet. Centrifuge at 12,000 x g for 5 minutes at 4°C.

25. Completely remove ALL of the supernatant from the pellet with a pipette.

26. Air-dry the pellet at least 10 minutes at room temperature.

27. Dissolve the plasmid DNA in a suitable volume (0.5 – 1.0 ml) of water or TE Buffer. DNA is immediately ready for downstream applications and may be moved to a smaller tube or diluted if desired.

6. Troubleshooting

6.1 Low yields of plasmid DNA

6.1.1 Temperature was too low

Temperature plays an important role in the isolation of optimal amounts of plasmid DNA. All solutions should be kept no cooler than room temperature (18 - 25°C).

6.1.2 Culture medium was not completely removed

Plasmid DNA will not bind optimally to the PhoenIX™ Giga Column unless it is at the correct salt concentration and pH. Excess culture media that is resuspended with the cells in the Cell Resuspension Buffer will have a significant negative impact on these critical parameters. Ensure all culture media is removed with a pipette prior to resuspending cells in step 4 of the protocol.

6.1.3 Alkaline lysis reagents were not added precisely

Plasmid DNA will not bind optimally to the PhoenIX™ Giga Column unless it is at the correct salt concentration and pH. Measure volumes of alkaline lysis reagents precisely to ensure best results.

6.1.4 DNA pellet was over-dried

Once a DNA pellet is over-dried, it can be difficult if not impossible to completely resuspend. Air-dry the pellet as directed in the protocol and do not use a vacuum pump system.

6.1.5 Variability in plasmid copy number

The total quantity of plasmid DNA in a particular *E. coli* host cell is influenced by many variables. The range of plasmid DNA per ml culture can vary from 0.2 µg/ml (“low copy”) to > 5.0 µg/ml (“high copy”). The size and sequence of specific DNA inserts will often influence the copy number of a particular plasmid; in some cases the copy number of a normally “high copy” plasmid will be reduced with a different insert.

6.2 Chromosomal DNA contamination

Chromosomal bacterial DNA is removed from the preparation by precipitation after the addition of the Neutralization Buffer and by subsequent filtration. This is only successful if shearing of the chromosomal DNA after cell lysis is kept to a minimum. Shearing of the chromosomal DNA occurs when the sample is vortexed after the addition of Lysis Buffer and/or Neutralization Buffer. Mix only by inversion after the addition of these solutions; do not vortex.

6.3 Multiple plasmid forms seen on agarose gel

A DNA band that runs slightly faster than the supercoiled plasmid DNA on a gel most likely represents irreversibly denatured plasmid DNA. Plasmid DNA will become irreversibly denatured if alkaline cell lysis (section 5, step 6) continues longer than the recommended 5 minutes.

6.4 RNA contamination

6.4.1 Preparation was at the incorrect salt concentration, pH or temperature

Due to the nature of the PhoenIX™ ion exchange resin, the separation of DNA and RNA is strongly dependent on the salt concentration, pH and temperature during binding, washing and elution. Follow the protocol exactly as directed and see sections 6.1.1 – 6.1 3 under "Low yields of plasmid DNA".

6.4.2 Sample was left on the column too long

Once the cleared bacterial lysate is added to the column, proceed with subsequent steps with no interruptions. Long pauses between steps may cause contamination of the purified plasmid DNA with small RNA species.

6.4.3 RNase A digestion was insufficient

Ensure the appropriate amount of RNase A was added to the Cell Resuspension Solution prior to beginning the protocol (see section 4.3). If the Cell Resuspension Solution was not stored at 4°C, or if it has been stored at 4°C for more than 6 months, new RNase A should be added prior to purifying more plasmid DNA.

Ensure the correct volume of prepared Cell Resuspension Solution was added in step 4 of the protocol.

6.4.4 Host strain may be naturally rich in RNA

Some host strains are extremely rich in RNA. Therefore, it is possible that a slight contamination of the plasmid DNA with residual RNA might occur, even in the presence of RNase. Increasing the volume of Neutralization Buffer in step 7 of the protocol by 10% can minimize contaminating RNA without significantly decreasing plasmid DNA yield.

7. Recommended Reference Format for Publications

"Plasmid DNA was purified using the PhoenIX™ Gigaprep Kit (Qbiogene, Inc., CA)".

8. Related Products

8.1 Plasmid Purification Kits

Purification Scale	Cat #	Kit Name	Size
Maxiprep	2075-300	PhoenIX™ Maxiprep Kit	25 preps
Midiprep	2075-200	PhoenIX™ Midiprep Kit	25 preps
Miniprep	2067-200	RapidPURE™ Plasmid Mini Kit	60 preps
	2067-400	RapidPURE™ Plasmid Mini Kit	120 preps
	2067-600	RapidPURE™ Plasmid Mini Kit	300 preps
	2067-200	RapidPURE™ Plasmid Mini 96 Kit	96 preps
	2067-400	RapidPURE™ Plasmid Mini 96 Kit	4 x 96 preps
	2069-400	Yeast RPM® Kit	100 preps
	2070-200	RPM® Kit	60 preps
	2070-400	RPM® Kit	120 preps
	2070-500	RPM® Kit	300 preps
	2070-600	RPM® Kit	600 preps
	2000-200	MiniPrep Express™ Matrix	1,250 preps
	2002-200	96well Prep Express	4 x 96 preps

8.2 Molecular Biology Certified™ Growth Media

BIO 101® Systems is well known for providing an excellent selection of high-quality growth media in a variety of formulations. We specialize in formulations for yeast and bacterial genetics, and offer more than a thousand recipes and variations. Each product is subjected to extensive quantitative testing, and is Molecular Biology Certified™ through qualitative, molecular biology based tests such as cell density and plasmid yield. Choose from a wide variety of convenient packaging formats: Pre-mixed powders are available in capsule form, single-use pouches, or in bulk sizes. Liquid media, pre-poured plates, and custom packaging options are also available. If you prefer to make your own media, BIO 101® Systems can supply you with raw materials such as tryptones and peptones, yeast extract, a wide variety of sugars and salts, antibiotics, and other media additives.

9. Product Use Limitation & Warranty

Unless otherwise indicated, this product is for research use only. Purchase of Qbiogene, Inc. products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. Qbiogene, Inc. makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and the sole liability of Qbiogene, Inc. hereunder shall be limited to, at our discretion, no replacement or compensation, product credits, refund of the purchase price of, or the replacement of materials that do not meet our specification. By acceptance of the product, Buyer indemnifies and holds Qbiogene, Inc. harmless against, and assumes all liability for, the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refund or replacement is conditioned on Buyer notifying Qbiogene, Inc. within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).

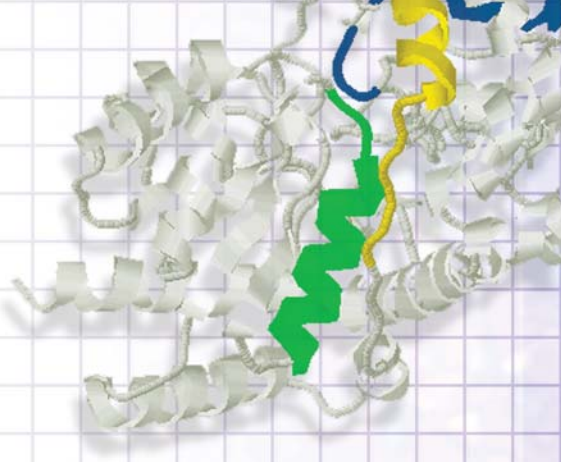
10. Trademarks and Patents

BIO 101® Systems and RPM® are registered trademarks of Qbiogene. MiniPrep Express™, Molecular Biology Certified™, PhoenIX™ and RapidPURE™ are trademarks of Qbiogene.

PhoenIX™ plasmid purification materials are covered under U.S. Patent # 5, 843, 312 and foreign equivalents

Appendix I: PhoenIX™ Gigaprep Quick Reference Protocol

Resuspend Pellet in Cell Resuspension Buffer (with RNase A)	125 ml
Add Lysis Buffer	125 ml
Add Neutralization Buffer	125 ml
Apply Lysate to Filtration Cartridge and Incubate	10 min
Apply Vacuum to Filtration Cartridge	1-2 min, ~20 inches Hg
Add Column Wash Buffer and Vacuum	50 ml
Prepare Giga Column with Equilibration Solution and Vacuum	200 ml
Apply Cleared Lysate to Giga Column and Vacuum	~400 ml
Add Column Wash Buffer and Vacuum	275 ml
(Optional) Repeat Wash	
Replace Collection Bottle and Add Elution Buffer	100 ml
Apply Soft Vacuum to Elute First 1/2 Volume	~5-8 inches Hg
Incubate without Agitation	1 min
Apply Soft Vacuum to Elute Second 1/2 Volume	~5-8 inches Hg
(Optional) Repeat Elution	
Add Isopropanol	0.7 volumes
Centrifuge	12,000 x g for 30 minutes at 4°C
Wash with 70% EtOH	20 ml
Centrifuge	12,000 x g for 5 minutes at 4°C
Air-dry pellet	10 minutes at room temp
Resuspend DNA	0.5 – 1.0 ml



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